

New International Patent Application
Gesellschaft für Biotechnologische Forschung mbH

Patent claims

1. An ssDNA molecule selected from the following group:

- (i) an ssDNA molecule having a sequence according to Figure 1;
- (ii) an ssDNA molecule which is 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% homologous to an ssDNA molecule according to (i) in respect of its number of nucleotides or its nucleotide sequence but which differs by at least one nucleotide from the ssDNA molecule according to (i) in respect of its number of nucleotides and/or its nucleotide sequence; and
- (iii) an ssDNA molecule having a sequence which is complementary to the sequence of an ssDNA molecule according to (i) or (ii).

2. A dsDNA molecule comprising an ssDNA molecule according to claim 1 and a strand complementary thereto.

3. An ssDNA molecule selected from the following group:

- (i) an ssDNA molecule having a sequence of positions 3.308 to 1 (ORF 16) of the sequence according to Figure 1;

- (ii) an ssDNA molecule having a sequence of positions 4706 to 3453 (ORF 15) of the sequence according to Figure 1;
- (iii) an ssDNA molecule having a sequence of positions 5719 to 7164 (ORF 14) of the sequence according to Figure 1;
- (iv) an ssDNA molecule having a sequence of positions 9557 to 7317 (ORF 13) of the sequence according to Figure 1;
- (v) an ssDNA molecule having a sequence of positions 12193 to 10550 (ORF 12) of the sequence according to Figure 1;
- (vi) an ssDNA molecule having a sequence of positions 12841 to 13881 (ORF 11) of the sequence according to Figure 1;
- (vii) an ssDNA molecule having a sequence of positions 14833 to 13835 (ORF 10) of the sequence according to Figure 1;
- (viii) an ssDNA molecule having a sequence of positions 14942 to 15586 (ORF 9) of the sequence according to Figure 1;
- (ix) an ssDNA molecule having a sequence of positions 15847 to 16983 (ORF 8) of the sequence according to Figure 1;

- (x) an ssDNA molecule having a sequence of positions 21154 to 18809 (ORF 7) of the sequence according to Figure 1;
- (xi) an ssDNA molecule having a sequence of positions 22366 to 23532 (ORF 6) of the sequence according to Figure 1;
- (xii) an ssDNA molecule having a sequence of positions 24591 to 26513 (ORF 5) of the sequence according to Figure 1;
- (xiii) an ssDNA molecule having a sequence of positions 26597 to 27517 (ORF 4) of the sequence according to Figure 1;
- (xiv) an ssDNA molecule having a sequence of positions 29858 to 30400 (ORF 3) of the sequence according to Figure 1;
- (xv) an ssDNA molecule having a sequence of positions 31220 to 32392 (TubA) of the sequence according to Figure 1;
- (xvi) an ssDNA molecule having a sequence of positions 33056 to 32397 (ORF 2) of the sequence according to Figure 1;
- (xvii) an ssDNA molecule having a sequence of positions 34195 to 33074 (TubZ) of the sequence according to Figure 1;

- (xviii) an ssDNA molecule having a sequence of positions 35422 to 34205 (ORF 1) of the sequence according to Figure 1;
- (xix) an ssDNA molecule having a sequence of positions 35522 to 40147 (TubB) of the sequence according to Figure 1;
- (xx) an ssDNA molecule having a sequence of positions 40144 to 48021 (TubC) of the sequence according to Figure 1;
- (xxi) an ssDNA molecule having a sequence of positions 48011 to 58558 (TubD) of the sequence according to Figure 1;
- (xxii) an ssDNA molecule having a sequence of positions 58551 to 62096 (TubE) of the sequence according to Figure 1;
- (xxiii) an ssDNA molecule having a sequence of positions 62103 to 70616 (TubF) of the sequence according to Figure 1;
- (xxiv) an ssDNA molecule which is hybridisable with a molecule according to (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), (x), (xi), (xii), (xiii), (xiv), (xv), (xvi), (xvii), (xviii), (xix), (xx), (xxi), (xxii) or (xxiii) under stringent conditions and especially has the same number of bases; and
- (xxv) an ssDNA molecule which is 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% homologous to an ssDNA molecule according to (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), (x), (xi), (xii), (xiii), (xiv), (xv), (xvi), (xvii), (xviii), (xix), (xx), (xxi), (xxii) or (xxiii) in respect of its number of nucleotides or its

nucleotide sequence but which differs by at least one nucleotide from that ssDNA molecule in respect of its number of nucleotides and/or its nucleotide sequence; and

- (xxvi) an ssDNA molecule having a sequence which is complementary to the sequence of a molecule according to (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), (x), (xi), (xii), (xiii), (xiv), (xv), (xvi), (xvii), (xviii), (xix), (xx), (xxi), (xxii), (xxiii), (xxiv) or (xxv).

4. A dsDNA molecule comprising an ssDNA molecule according to claim 3 and a strand complementary thereto.

5. An ssDNA molecule selected from the following group:

- (i) an ssDNA molecule having a sequence of positions 35747 to 36769 (domain C of the tubB gene) of the sequence according to Figure 1;
- (ii) an ssDNA molecule having a sequence of positions 37184 to 39817 (domain A of the tubB gene) of the sequence according to Figure 1;
- (iii) an ssDNA molecule having a sequence of positions 38369 to 39730 (domain NMT of the tubB gene) of the sequence according to Figure 1;
- (iv) an ssDNA molecule having a sequence of positions 39818 to 40069 (domain PCP of the tubB gene) of the sequence according to Figure 1;

- (v) an ssDNA molecule having a sequence of positions 40372 to 41397 (domain C of the tubC gene) of the sequence according to Figure 1;
- (vi) an ssDNA molecule having a sequence of positions 41824 to 43215 (domain A of the tubC gene) of the sequence according to Figure 1;
- (vii) an ssDNA molecule having a sequence of positions 43216 to 43461 (domain PCP of the tubC gene) of the sequence according to Figure 1;
- (viii) an ssDNA molecule having a sequence of positions 43552 to 44574 (domain C of the tubC gene) of the sequence according to Figure 1;
- (ix) an ssDNA molecule having a sequence of positions 44980 to 47631 (domain A of the tubC gene) of the sequence according to Figure 1;
- (x) an ssDNA molecule having a sequence of positions 46153 to 47547 (domain NMT of the tubC gene) of the sequence according to Figure 1;
- (xi) an ssDNA molecule having a sequence of positions 47632 to 47868 (domain PCP of the tubC gene) of the sequence according to Figure 1;
- (xii) an ssDNA molecule having a sequence of positions 48011 to 49321 (domain KS of the tubD gene) of the sequence according to Figure 1;

- (xiii) an ssDNA molecule having a sequence of positions 49622 to 50584 (domain AT of the tubD gene) of the sequence according to Figure 1;
- (xiv) an ssDNA molecule having a sequence of positions 51473 to 52309 (domain KR of the tubD gene) of the sequence according to Figure 1;
- (xv) an ssDNA molecule having a sequence of positions 53066 to 53980 (domain ER of the tubD gene) of the sequence according to Figure 1;
- (xvi) an ssDNA molecule having a sequence of positions 54158 to 54460 (domain ACP of the tubD gene) of the sequence according to Figure 1;
- (xvii) an ssDNA molecule having a sequence of positions 54461 to 55870 (domain HC of the tubD gene) of the sequence according to Figure 1;
- (xviii) an ssDNA molecule having a sequence of positions 56000 to 57412 (domain A of the tubD gene) of the sequence according to Figure 1;
- (xix) an ssDNA molecule having a sequence of positions 57413 to 57643 (domain PCP of the tubD gene) of the sequence according to Figure 1;
- (xx) an ssDNA molecule having a sequence of positions 58689 to 59714 (domain C of the tubE gene) of the sequence according to Figure 1;

- (xxi) an ssDNA molecule having a sequence of positions 60156 to 61697 (domain A of the tubE gene) of the sequence according to Figure 1;
- (xxii) an ssDNA molecule having a sequence of positions 61698 to 61967 (domain PCP of the tubE gene) of the sequence according to Figure 1;
- (xxiii) an ssDNA molecule having a sequence of positions 62127 to 63320 (domain KS of the tubF gene) of the sequence according to Figure 1;
- (xxiv) an ssDNA molecule having a sequence of positions 63711 to 64676 (domain AT of the tubF gene) of the sequence according to Figure 1;
- (xxv) an ssDNA molecule having a sequence of positions 64959 to 65882 (domain KR of the tubF gene) of the sequence according to Figure 1;
- (xxvi) an ssDNA molecule having a sequence of positions 65985 to 67061 (domain CMT of the tubF gene) of the sequence according to Figure 1;
- (xxvii) an ssDNA molecule having a sequence of positions 67242 to 67829 (domain DH of the tubF gene) of the sequence according to Figure 1;
- (xxviii) an ssDNA molecule having a sequence of positions 68247 to 69128 (domain ER of the tubF gene) of the sequence according to Figure 1;

- (xxix) an ssDNA molecule having a sequence of positions 69360 to 69605 (domain PCP of the tubF gene) of the sequence according to Figure 1;

- (xxx) an ssDNA molecule having a sequence of positions 69759 to 70586 (domain TE of the tubF gene) of the sequence according to Figure 1;

- (xxxi) an ssDNA molecule which is hybridisable with a molecule according to (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), (x), (xi), (xii), (xiii), (xiv), (xv), (xvi), (xvii), (xviii), (xix), (xx), (xxi), (xxii), (xxiii), (xxiv), (xxv), (xxvi), (xxvii), (xxviii), (xxix) or (xxx) under stringent conditions and especially has the same number of bases;

- (xxxii) an ssDNA molecule which is 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% homologous to an ssDNA molecule according to (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), (x), (xi), (xii), (xiii), (xiv), (xv), (xvi), (xvii), (xviii), (xix), (xx), (xxi), (xxii), (xxiii), (xxiv), (xxv), (xxvi), (xxvii), (xxviii), (xxix) or (xxx) in respect of its number of nucleotides or its nucleotide sequence but which differs by at least one nucleotide from that ssDNA molecule in respect of its number of nucleotides and/or its nucleotide sequence; and

- (xxxiii) an ssDNA molecule having a sequence which is complementary to the sequence of a molecule according

to (i), (ii), (iii), (iv), (v), (vi), (vii), (viii), (ix), (x), (xi), (xii), (xiii), (xiv), (xv), (xvi), (xvii), (xviii), (xix), (xx), (xxi), (xxii), (xxiii), (xxiv), (xxv), (xxvi), (xxvii), (xxviii), (xxix), (xxx), (xxxi) or (xxxii).

6. A dsDNA molecule comprising an ssDNA molecule according to claim 5 and a strand complementary thereto.

7. Variants or mutants which result from a substitution, insertion or deletion of nucleotides or from an inversion of nucleotide segments of an ssDNA molecule according to claim 1, 3 or 5 or of a dsDNA molecule according to claim 2, 4 or 6, those variants and mutants encoding enzyme variants or enzyme mutants for the production of secondary substance(s) having properties characteristic of tubulysins.

8. RNA

- (a) having a sequence corresponding to that of an ssDNA molecule according to one of claim 1, 3, 5 or 7 or
 - (b) having a sequence of an RNA according to (a) but in the opposite direction (anti-sense), or
 - (c) having a sequence of an RNA according to (a) or (b) and having a strand complementary thereto,
- in each case optionally as an element of a recombinant vector.

9. A vector, especially an expression vector, having a DNA molecule according to one of claims 1 to 7.

10. A cell, especially for expression, into which a DNA molecule according to one of the preceding claims or a vector according to claim 8 or 9 has been integrated.

11. A cell according to claim 10, the cell being derived from culturable bacteria, especially Myxobacteria, preferably Angiococcus, especially A. disciformis, Archangium, especially A. gephyra, Escherichia coli, pseudomonads or actinomycetes.

12. Use of a vector according to claim 8 or 9 for the transformation of cells or organisms for the transient or permanent expression of one or more proteins (expression product(s) which is/are encoded by a DNA (ssDNA or dsDNA) of the vector).

13. Use of a cell according to claim 10 or 11 for the enzymatic biosynthesis, metasyntesis or partial synthesis of a tubulysin, especially tubulysin A, B, C, D, E and/or F.

14. An expression product of a DNA molecule according to one of claims 1 to 7 or of a vector according to claim 8 or 9 or of a cell according to claim 10 or 11.

15. A polynucleotide comprising a sequence as defined in SEQ ID NO: 1, 18, 33 or 36, or a fragment thereof.

16. A polynucleotide according to claim 15, wherein the fragment is a CDS defined in the sequence protocol.

17. A vector comprising a polynucleotide according to claim 15 or 16.

18. A cell comprising a vector according to claim 17.

19. A polypeptide comprising at least one sequence as defined in SEQ ID NO: 2 to 17, 19 to 32, 34, 35, 37 and/or 38 and/or a fragment and/or derivative thereof.

20. Use of at least one polynucleotide as defined in SEQ ID NO: 1, 18, 33 and/or 36 and/or at least one fragment thereof and/or at least one polypeptide as defined in SEQ ID NO: 2 to 17, 19 to 32, 34, 35, 37 and/or 38 and/or at least one fragment thereof in the production of a pharmaceutical composition for the treatment of undesirable cell growth or undesirable cell proliferation in an individual.

21. Use according to claim 20, wherein the undesirable cell growth or undesirable cell proliferation is a tumour.

22. Use according to claim 20, wherein the undesirable cell growth is a pathogenic infection.

23. Use according to claim 22, wherein the pathogenic infection is a mycosis, malaria or a parasitic disease.

24. A pharmaceutical composition comprising at least one polynucleotide as defined in SEQ ID NO: 1, 18, 33 and/or 36 and/or at least one fragment thereof and/or at least one polypeptide as defined in SEQ ID NO: 2 to 17, 19 to 32, 34, 35, 37 and/or 38 and/or at least one fragment thereof.

25. A pharmaceutical composition according to claim 24, which further comprises at least one pharmaceutically acceptable carrier.

26. A method of producing tubulysins and tubulysin biosynthesis proteins, comprising the steps:

- (a) expression of at least one polynucleotide as defined in SEQ ID NO: 1, 18, 33 and/or 36 and/or at least one fragment thereof and/or at least one polypeptide as defined in SEQ ID NO: 2 to 17, 19 to 32, 34, 35, 37 and/or 38 and/or at least one fragment thereof, and
- (b) purification of the expression products.

27. A method according to claim 26, wherein expression is carried out in prokaryotic or eukaryotic cells and/or by *in vitro* expression.

28. A method of finding genes which are involved in the biosynthesis of tubulysins, comprising the steps:

- (a) hybridisation of at least one polynucleotide as defined in SEQ ID NO: 1, 18, 33 and/or 36 and/or at least one fragment thereof with DNA, RNA and/or cDNA of a species that is not identical to *Angiococcus disciformis*, and
- (b) isolation and characterisation of the hybridised DNA, RNA and/or cDNA.

29. A kit for the production of tubulysins, comprising:

- (a) at least one polynucleotide according to claim 15 or 16 and/or at least one vector according to claim 17
or
- (b) suitable media and buffers for the multiplication of cells which allow expression of the polynucleotide and/or vector and
- (c) suitable means for purification of the expression product(s).

30. Use of a composition comprising at least one polypeptide as defined in SEQ ID NO: 2 to 17, 19 to 32, 34, 35, 37 and/or 38 and/or at least one biologically active fragment or derivative thereof as a disinfectant.

31. Use according to claim 30, wherein the composition is liquid or in powder form.

32. A disinfectant as defined in claim 30 or 31.